

Further Evidence for Pre-metamorphosis Larval Eye Reduction in the Holometabola (Insecta: Mecoptera: *Panorpa vulgaris* IMHOFF & LABRAM, 1836)

With 3 figures and 2 tables

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Summary

Larvae of *Panorpa* are eye developmentally unusual. They possess a multi-lense complex-eye-like visual organ, which was found not to be part of the later adult eye. A highly significant deviation in the ommatidia number during the larval stages was found, with instar 3 (of 4) having most ommatidia.

Key words

Eye, Mecoptera, *Panorpa*, Metamorphosis, Development

Zusammenfassung

Die Larven der Gattung *Panorpa* weisen eine ungewöhnliche Augenentwicklung auf. Sie besitzen ein aus vielen Linsen bestehendes, komplexaugenähnliches Organ, welches nicht zu einem Teil des späteren Adultauges wird. Die einzelnen Larvenstadien unterscheiden sich hochsignifikant in der Anzahl der Ommatidien: das dritte von vier Larvenstadien besitzt die meisten Ommatidien.

Introduction

No matter what the actual phylogenetic grouping amongst the Mecoptera is, they are considered to be less derived in respect to eye development than other insects (FRIEDRICH et al. 2006). Beside the remarkably facet-eye-like organisation of the larval eyes (see Fig. 1), which sets them clearly apart from simple stemmata-possessing groups, their less derived state of eye development marks them as an excellent object for the study of eye development in general. The first aim was to show by histology and TEM, which both can only provide isolated information about one moment during development, what happens to the components, i. e., the cells and the organelles of the larval eye, once the metamorphosis sets in. The second was to re-check previous reports of a constant number of omma-

tidia during post-embryonal development (BUSCHBECK et al. 2008; FRIEDRICH 2006; FRIEDRICH 2008; FRIEDRICH et al. 2011; PAULUS 1979; PAULUS 1989) considering new data on developmental changes eg. in Thysanoptera (KUMM 1997) or Coleoptera (FRIEDRICH et al. 2006; FRIEDRICH et al. 2011; SCHULZ et al. 1984).

The blueprint of a typical insect ommatidium – be it holo- or hemimetabolous in origin

Typically an insect ommatidium consists of eight retinular cells, four sempercells and two main pigment cells (ANDERSON 1978; MELZER et al. 2000; NOWEL 1981). In the case

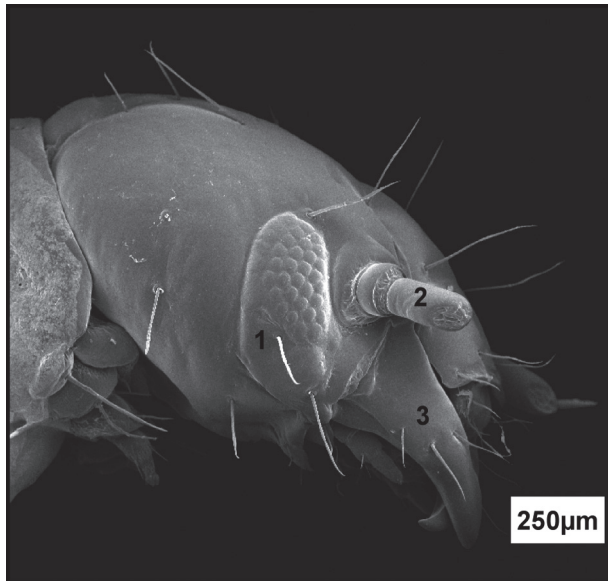


Fig. 1: SEM side view of a 3rd instar larvae head and eye, notice the well defined rows of ommatidia. Figure annotations: 1: eye; 2: antenna; 3: mandible.

of a hemimetabolous development a sequential adding of new ommatidia adjacent to older ones takes place leading to adults with higher ommatidia number than the larva (ANDERSON 1978; FRIEDRICH 2006; MEINERTZHAGEN 1973; NOWEL 1981; PAULUS 1979; PAULUS 1989).

In the holometabolous development, the embryonal eye development is partly suppressed until metamorphosis and conducted in cell invaginations, which are developmentally independent from JH (juvenile hormone) (MEINERTZHAGEN 1973; MEINERTZHAGEN 1975; TRUMAN et al. 1999; TRUMAN et al. 2002). The development starts with the R8 (R=Retinularcell), followed by the so called five-cell-cluster (R58+R2/5+R3/4) and is completed to the usual eight-cell-cluster by adding the R1/6 and R7 cells (FRIEDRICH et al. 2011; SALZER et al. 2009; TOMLINSON 1988; WOLFF et al. 1993).

The development follows a morphogenic furrow, which sweeps from posterior to anterior, initiation cell proliferation and differentiation (CHAMPLINE et al. 1998; EGELHAAF et al. 1988; FRIEDRICH et al. 2011; MEINERTZHAGEN 1975; READY 1989; WOLFF et al. 1993; WOLPERT 1969). Thus the basic difference between holometabola and hemimetabola regarding eye development is continuous growth with addition of ommatidia in hemimetabola and de novo development in the metamorphic pupal stage following a developmental grading in holometabola.

State-of-the-art knowledge regarding eye development in *Panorpa*

Although some species have recently been investigated with TE-Microscopy eg. *Panorpa dubia* CHOU & WANG,

1981 and *Panorpodes kuandianensis* ZHONG, ZHANG & HUA, 2011 by CHEN and colleagues (CHEN et al. 2012; CHEN et al. 2013) or even *Panorpa vulgaris* by MELZER (1994) no coherent developmental model has surfaced yet. The problematics have already been keenly identified by BIERBRODT back in the 1940s. She stated that the actual developmental step takes place in the early pre-puppa, which weren't at her disposal (BIERBRODT 1942). The larval ommatidium consists of one biconvex lense, two primary pigment cells, four sempercells and eight retinular cells in two layers, this overall structure has been deemed highly conserved by Friedrich und Jackowska (FRIEDRICH et al. 2006; PAULUS 1979). According to LAND there are only two ways to optimize optical resolution in an eye: 1st enlarging the eye while keeping the same receptor density - the solution in vertebrates -, or 2nd multiplication of small eye units - the solution realized in arthropod eyes (LAND et al. 2002).

All comes down to the question whether or not the stemmata of the holometabola are an autapomorphic feature. In this context it would be interesting to know whether stemmata are secondarily simplified adult eyes leading to the complex eyes of larva being a tertiary variation on stemma, this is the thesis favoured in literature (BEUTEL et al. 2010; FRIEDRICH et al. 2006; PAULUS 1979; PAULUS 1986a, 1989b; KRISTENSEN 1995; MELZER et al. 1989) but it is also occasionally contradicted, eg. BEUTEL, who questions the polarity of the compound eye character state (BEUTEL et al. 2006). Alternatively they could be a completely new development or the stemma might be a secondary reduction which happened after the emergence of holometabola, not necessarily individually but in different groups, this is clearly less likely. Which alternative best explains the stemmata could be tested by verifying the situation in the eye development of less derived groups which lead FRIEDRICH et al. to the statement „To confirm this idea, it will be necessary... to study the cellular dynamics of larval and adult eye development in the less derived visual systems of holometabola species such as scorpion flies (Mecoptera)“ (FRIEDRICH et al. 2006).

Materials & Techniques

Animal obtention

All animals were taken of my own breeding stock. *Panorpa vulgaris* was kept and reared according to the guidelines provided by ROTTMAR (1966) with the exception that fish food (white bloodworms) was substituted for the advised feeding.

Counting method

20 head capsula of previously sedated, decapitated and fixed animals per instar have been counted using a Zeiss AxioCam MRc5 mounted on a Leica MZ125 binocular for documentation. Each individual was counted three times and averaged.

Statistics

Using SPSS 21, the acquired data of ommatidia number of twenty individuals per instar have been analysed regarding equal distribution with Shapiro-Wilk-Test and regarding equal variance with Levene Statistic. According to the unequal distribution of one group (instar 4) further analysis was conducted non-parametrically with Kruskal-Wallis-Test followed up by a pairwise comparison with Mann-Whitney-U-Tests. Significance values have been conservatively Bonferroni corrected.

Histology

5 µm thick paraffin sections and 1,5 µm semi-thin sections (embedded in hard araldid colored with Stevenes Blue) were produced to complement the TEM studies.

Fixation

Animals for semi- and ultrathin sections were glutaraldehyde and osmiumtetroxide fixated. For the histological paraffin sections the specimens were fixed with Formol-Acetic Acid-Ethanol.

Results

Histology & TEM

In both histology and the TEM analyses it was possible to identify new as well as degrading larval ommatidia (see Fig. 2).

Statistics

The results of the statistic analyze of ommatidia number differences are summarized in Fig. 3, Tab. 1 and Tab. 2.

Tab. 1: Shapiro-Wilk and Levene Statistic and confidence intervals for instar 1-4. Singling the 4th larval stage out for not being evenly distributed.

Tests and test groups if applicable	Test statistic	Significance values
Levene Statistic based on mean (Median)	1,530 (1,489) df1:3; df2:76	0,214 (0,224)
Shapiro-Wilk Statistic 1	0,950 df:20	0,367
Shapiro-Wilk Statistic 2	0,916 df:20	0,084
Shapiro-Wilk Statistic 3	0,969 df:20	0,723
Shapiro-Wilk Statistic 4	0,766 df:20	<0,001
confidence intervals	lower border	upper border
L1	28,83	31,50
L2	29,32	33,25
L3	34,15	37,05
L4	31,24	33,63

Discussion

Histology & TEM

Whereas only one individual was investigated in TEM, a pigment spot could be found many times in the histological sections, and also is mentioned in literature (BIERBRODT 1942; ROTTMAR 1966) (unpublished

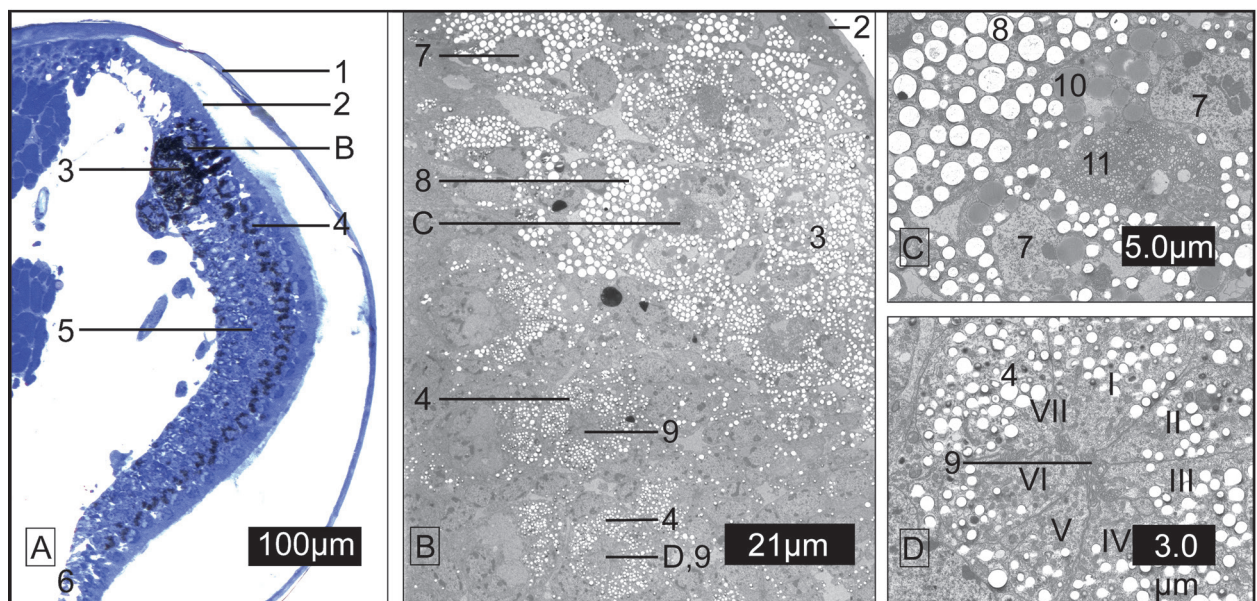


Fig. 2: A–D: A: eye segment of a transversal section through the first pupal stage head of *Panorpa vulgaris*, upper half of the picture dorsal, lower half ventral; B: TEM overview of the area of the pigment spot from the same specimen; C: detailed TEM picture of a degrading rhabdomer; D: detailed TEM picture of a developing rhabdomer.

Figure annotations: B: position of detail shown in part B; C: position of detail shown in part C; D: position of detail shown in part D; 1: pupal cuticula; 2: developing adult cuticula; 3: pigment spot; 4: area encircled by pigment vesicles; 5: part of differentiation zone; 6: border of proliferation zone; 7: degrading nucleus; 8: large pigment vesicles; 9: group of rhabdomer building reticular cells; 10: lipid vesicles aggregation sign of degradation; 11: degrading larval rhabdomer; I–VII: new developing reticular cells, numbered.

Tab. 2: Kruskal-Wallis-Test and follow-up pairwise Mann-Whitney-U-Test in brackets the non-Bonferroni-corrected values are provided as well.

Stage	Mean	St Error (for N=20)
L1	31,17	± 0,64
L2	31,28	± 0,94
L3	35,6	± 0,69
L4	31,62	± 0,96
Sign. Comp.	K-W-Test	<0,001 df3 Chi ² : 22,29
	M-W-U-Test	M-W-U-Test U Statistic
L3:L1	a < 0,01	38
L3:L2	a < 0,01	82
L3:L4	a < 0,01	64,5

data). The histology data support the hypothesis on the degradation of the larval eye structures and the new development of adult eye ommatidia, as expected for a holometabolous insect. Since ommochromes cannot be metabolized (KAYSER 1979; LINZEN 1974) it remains unclear whether they are excreted or recycled, a precedent for the latter exists in Thysanoptera (KUMM 1997). It seems neither to be a case like in the Chaoboridae where the adult eyes develop early (BRAMMER 1970; MELZER et al. 1991; MELZER et al. 1994a; MELZER 2009) nor like in the Nannochoristidae with more than one eye in the larval stage (MELZER et al. 1994b).

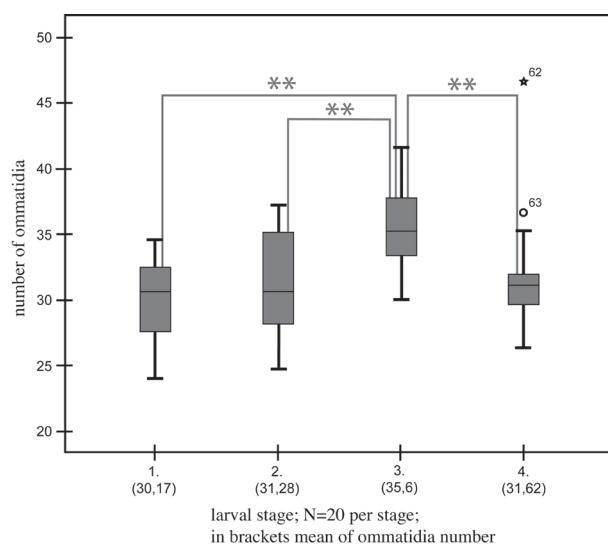


Fig. 3: Means of all four instars shown as Boxplots, significances are denoted as following * significant ($\alpha < 0,05$), ** highly significant ($\alpha < 0,01$).

Histology could also show that one ommatidium is below one "Cornea lense", no fusion of ommatidia was found. Fusion of ommatidia is sometimes reported for larval eyes of other groups (BEUTEL et al. 2008; LIU et al. 2004; MANDAPAKA et al. 2006).

That Mecoptera possess true compound eyes if one follows a functional definition is in accordance with observations by GILBERT (1994).

Placement of statistic results

The 3rd instar has significantly more ommatidia than the other three stages. Regarding the beginning of eye reduction there is a precedent in Coleoptera (FRIEDRICH et al. 2006; FRIEDRICH et al. 2011) and *Drosophila* (KENYON et al. 2003; KUMAR 2001; KUMAR et al. 2001b, 2001c). The fact that large instars have not only larger ommatidia but also more of them resembles a hemimetabolous development, but then Mecoptera are thought to be less derived in eye development than other holometabolous groups (FRIEDRICH 2006). An increasing number of larval ommatidia clearly contradicts earlier notions (BUSCHBECK et al. 2008; PAULUS 1989).

Outlook

Having shown this for *Panorpa vulgaris* with histology and statistics a broader, more general approach within the *Panorpa* relationship would be desirable. With the genomic tools available today referring to 1000K project and the explicit knowledge of model organisms like *Drosophila* and red flour beetle genetics (DANIEL et al. 1999; ELLIS et al. 1993; FRIEDRICH 2003; FRIEDRICH et al. 1996; FRIEDRICH et al. 2000; GEHRING 2002; HAYNIE et al. 1986; HELFRICH-FÖRSTER et al. 2002; KLINGLER 2004; KOJIMA et al. 1991; KUMAR 2001; KUMAR et al. 2001a, 2001b, 2001c; MEINERTZHAGEN 1973; MEINERTZHAGEN 1989; MOSES et al. 1991; OSORIO 2007; PAPPU et al. 2004; PLAUTZ et al. 1996; QUIRING et al. 1994; SEN 2006; SERIKAKU et al. 1994; STEINBERG 1941; SUZUKI et al. 2000; WOLFF et al. 1993; YASUYAMA et al. 1999) a molecular approach to in-vivo cell tracking might be feasible and probably rewarding. With this approach it would also be possible to track possible remains of the larval eye later on, which might be as small as 4 unpigmented cells if we consider the example of the Bolwig organ transformation into the Hofbauer-Buchner eyelet (BOLWIG 1946; FRIEDRICH 2008; HOFBAUER et al. 1989; HELFRICH-FOERSTER et al. 2002).

Beside either a ecological study of the larval behavior or an investigation of the cornea lenses' refraction properties as done for Scarab Beetles by MCINTYRE (1985) could provide useful insight into the actual usage of the eyes by these burrowing animals.

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